Figure 1

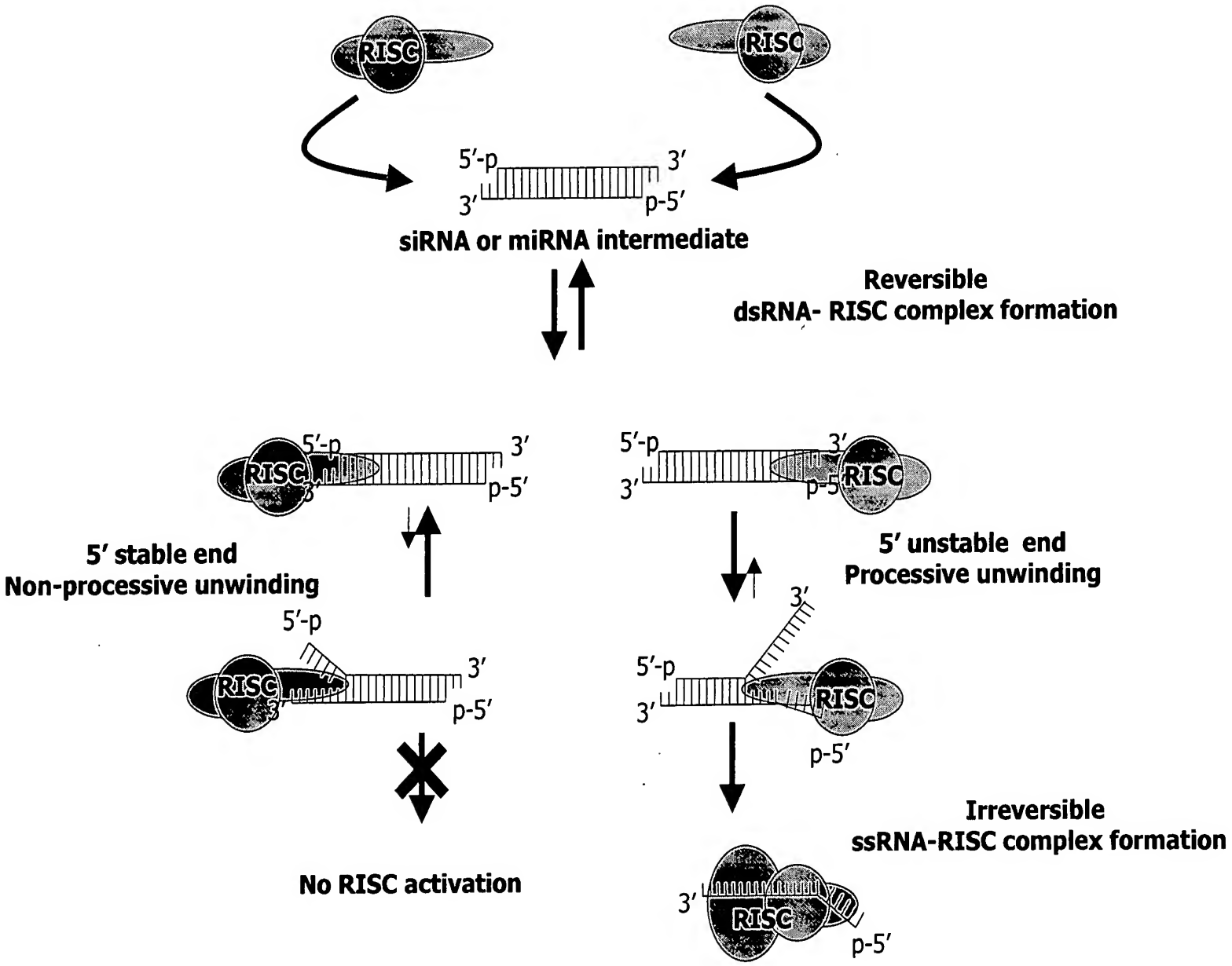

RISC
RISC
5′-p
3′
3′
p-5′
siRNA or miRNA intermediate
Reversible
dsRNA- RISC complex formation
5′-p
3′
RISC
p-5′
5′ stable end
Non-processive unwinding
5′-p
3′
p-5′
RISC
5′ unstable end
Processive unwinding
5′-p
3′
RISC
3′
5′-p
3′
RISC
p-5′
No RISC activation
Irreversible
ssRNA-RISC complex formation
3′
RISC
p-5′

Figure 2

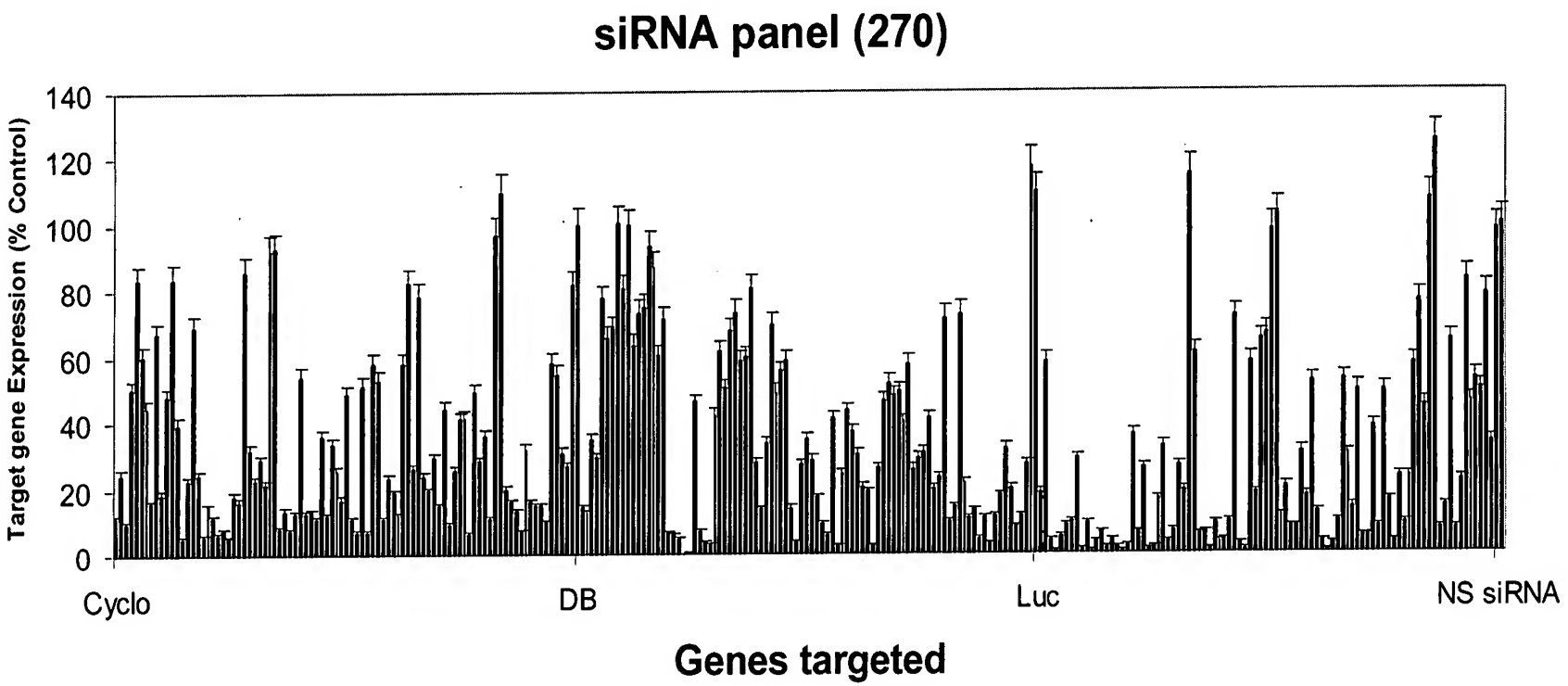

siRNA panel (270)
Target gene Expression (% Control)
140
120
100
80
60
40
20
0
Cyclo
DB
Luc
NS siRNA
Genes targeted

Figure 3a

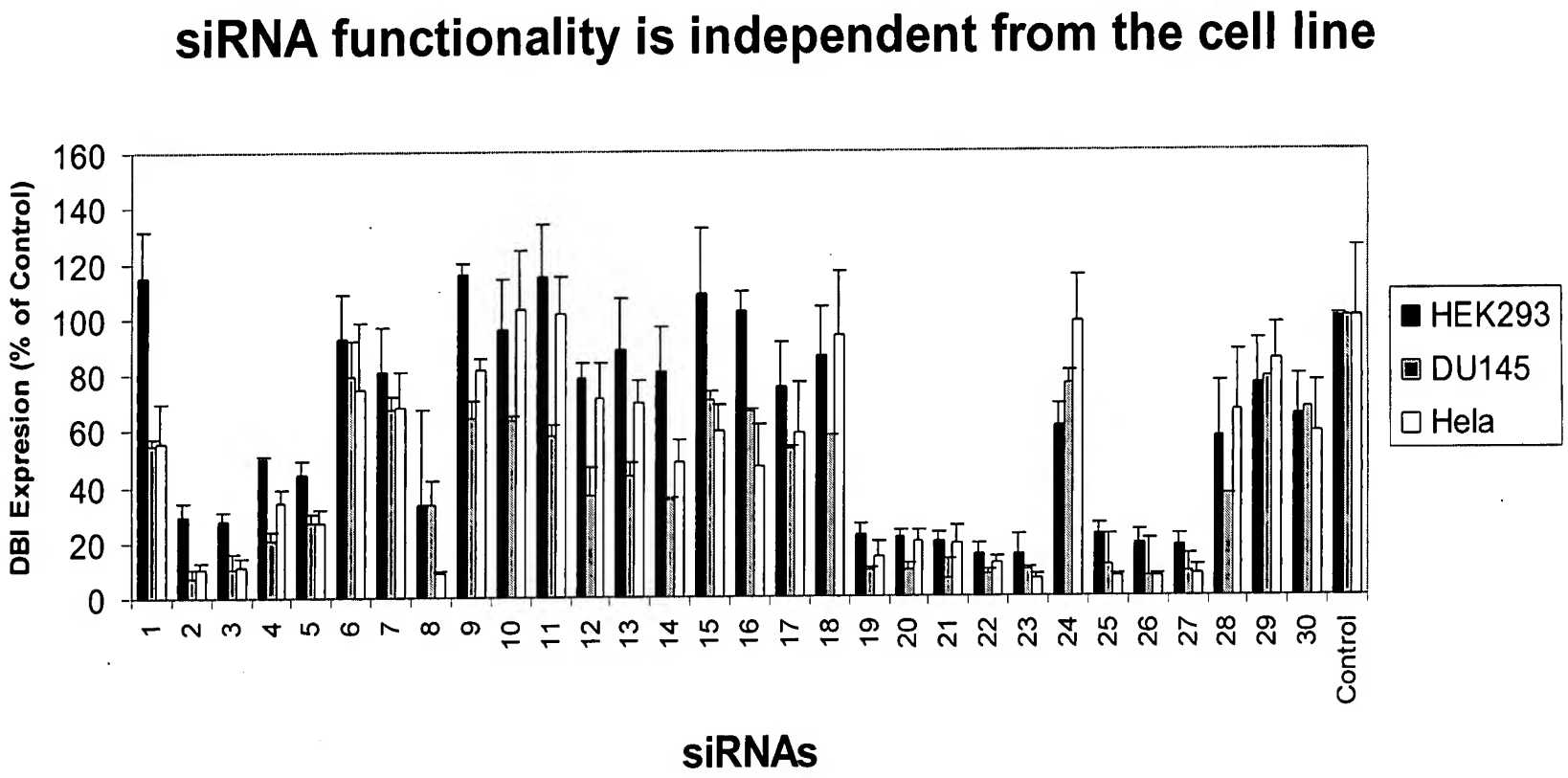

siRNA functionality is independent from the cell line
DBI Expresion (% of Control)
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Control
siRNAs
HEK293
DU145
Hela

Figure 3b

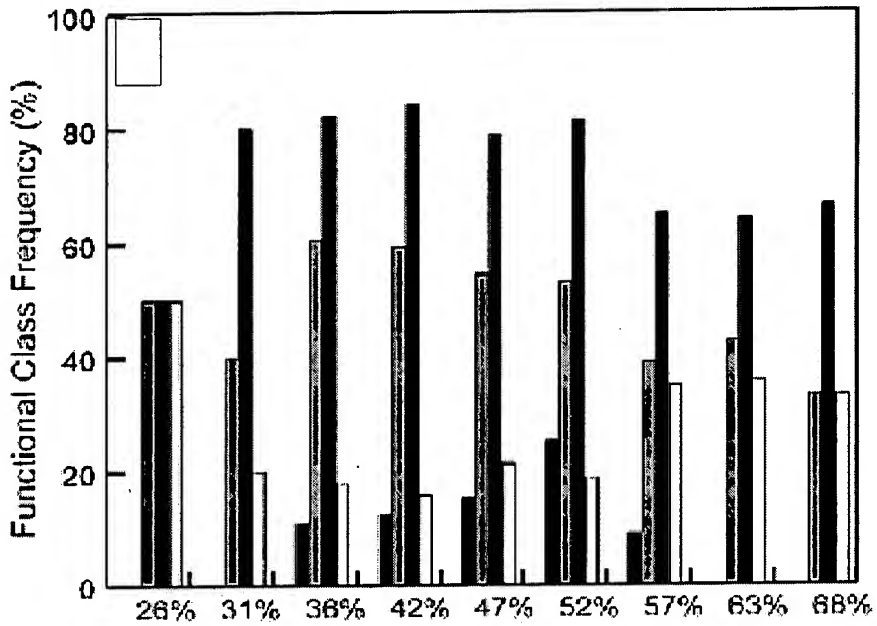


Figure 3c

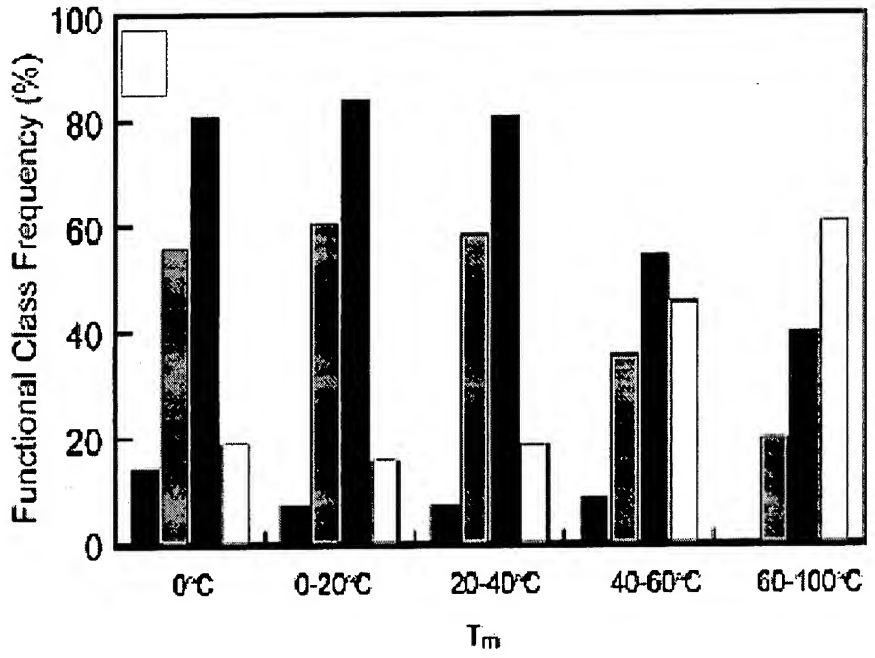


Figure 4

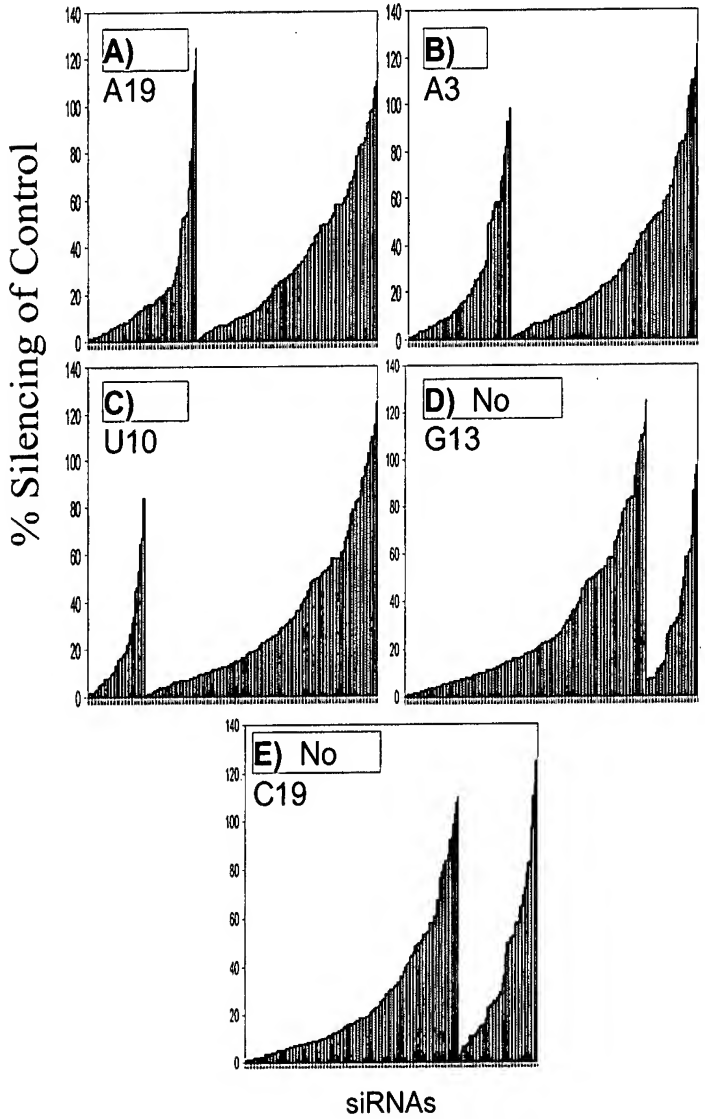


Figure 5A

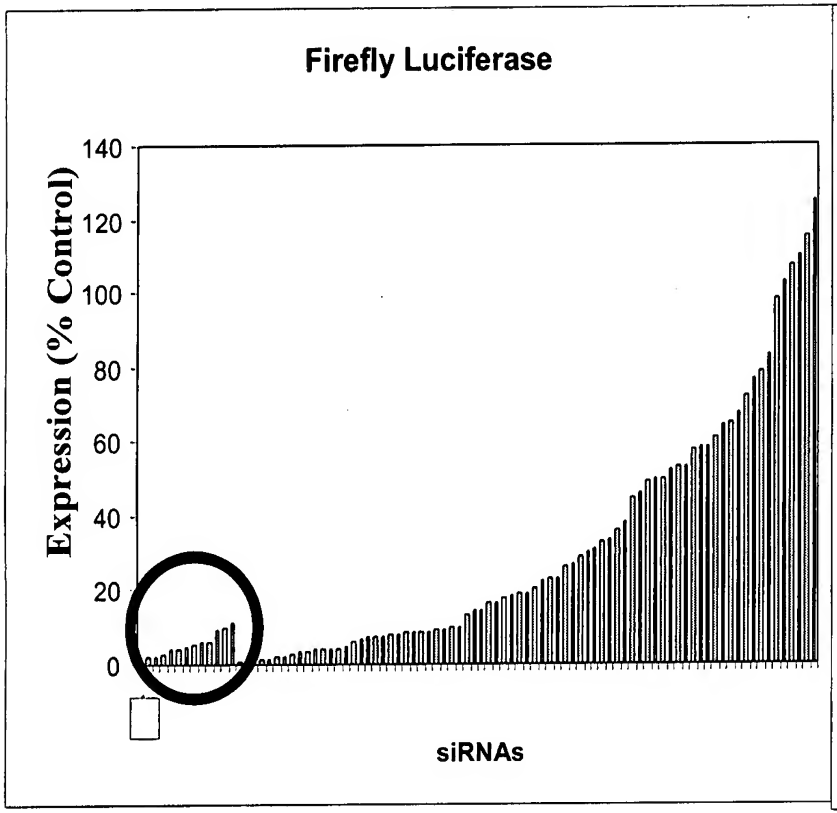


Figure 5B

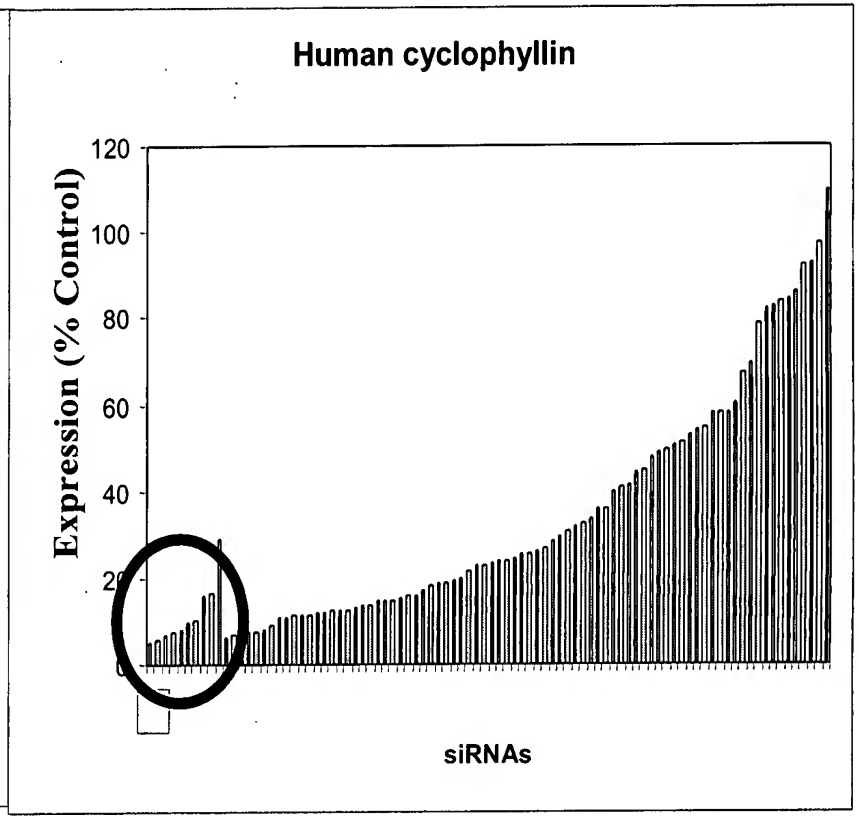


Figure 6a

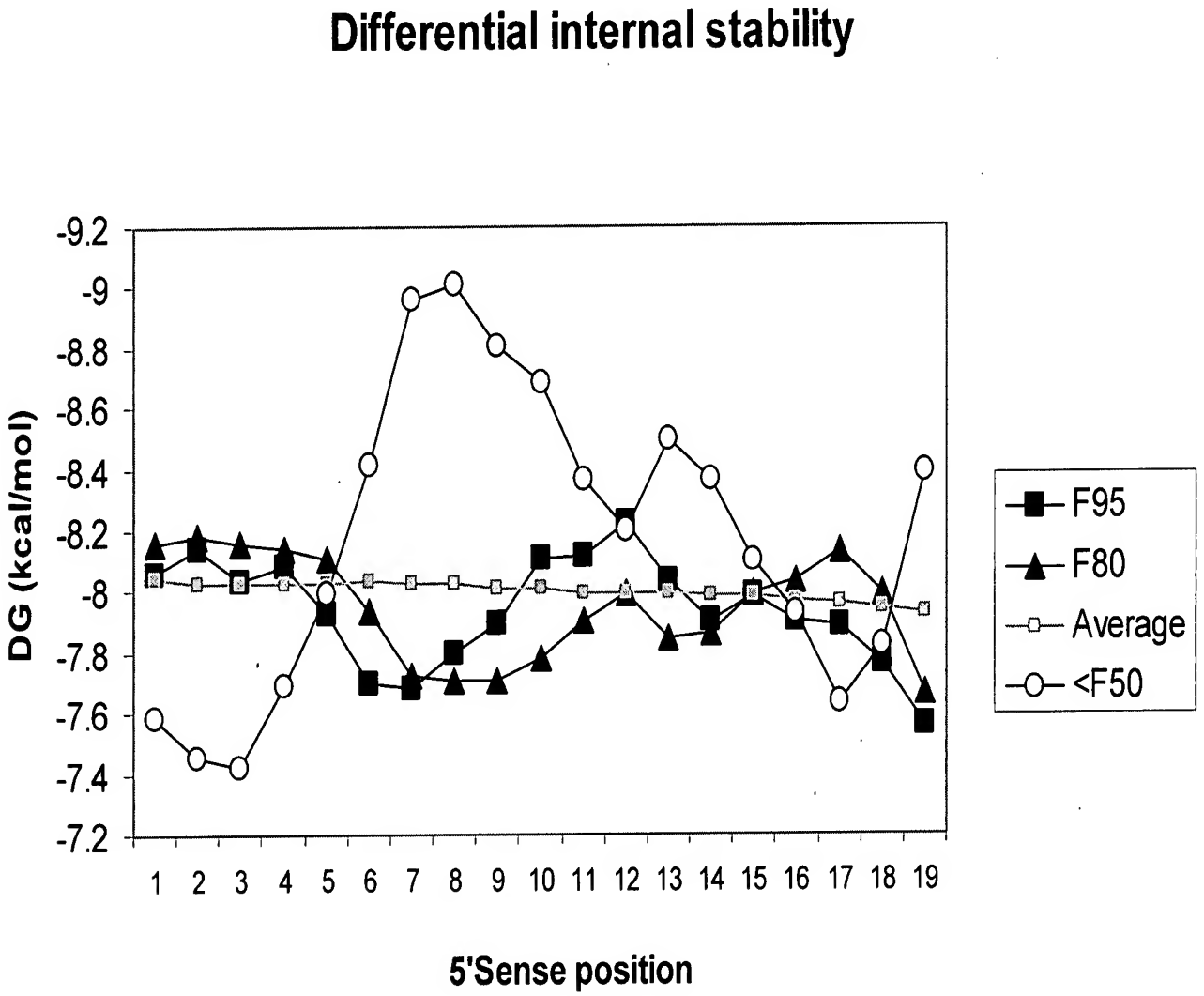

Differential internal stability
DG (kcal/mol)
-9.2
-9
-8.8
-8.6
-8.4
-8.2
-8
-7.8
-7.6
-7.4
-7.2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
5'Sense position
F95
F80
Average
<F50

Figure 6b

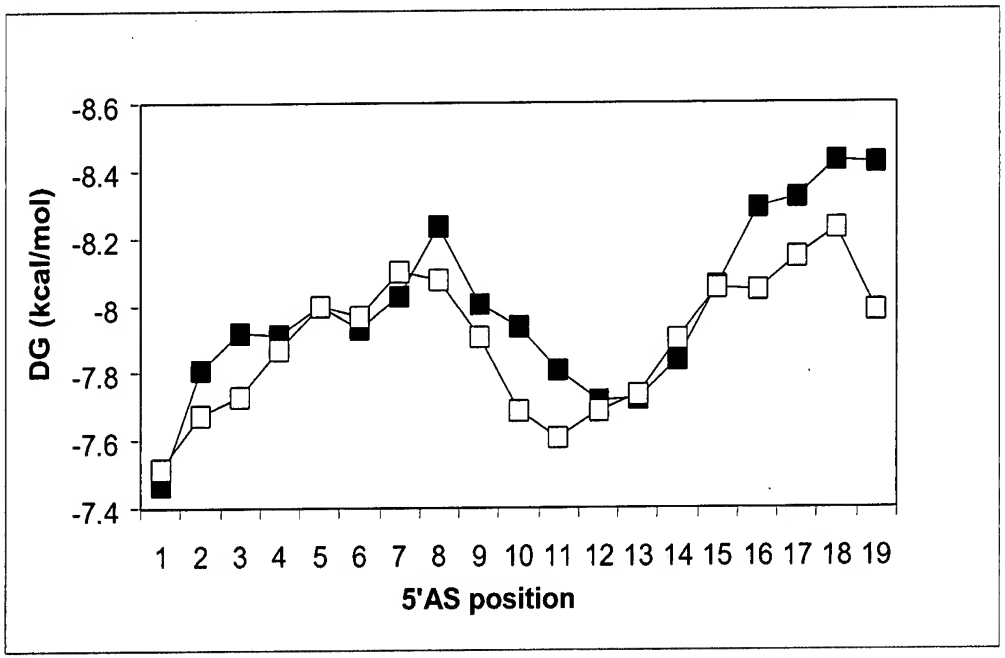

-8.6
-8.4
-8.2
-8
-7.8
-7.6
-7.4
DG (kcal/mol)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
5'AS position

Figure 7

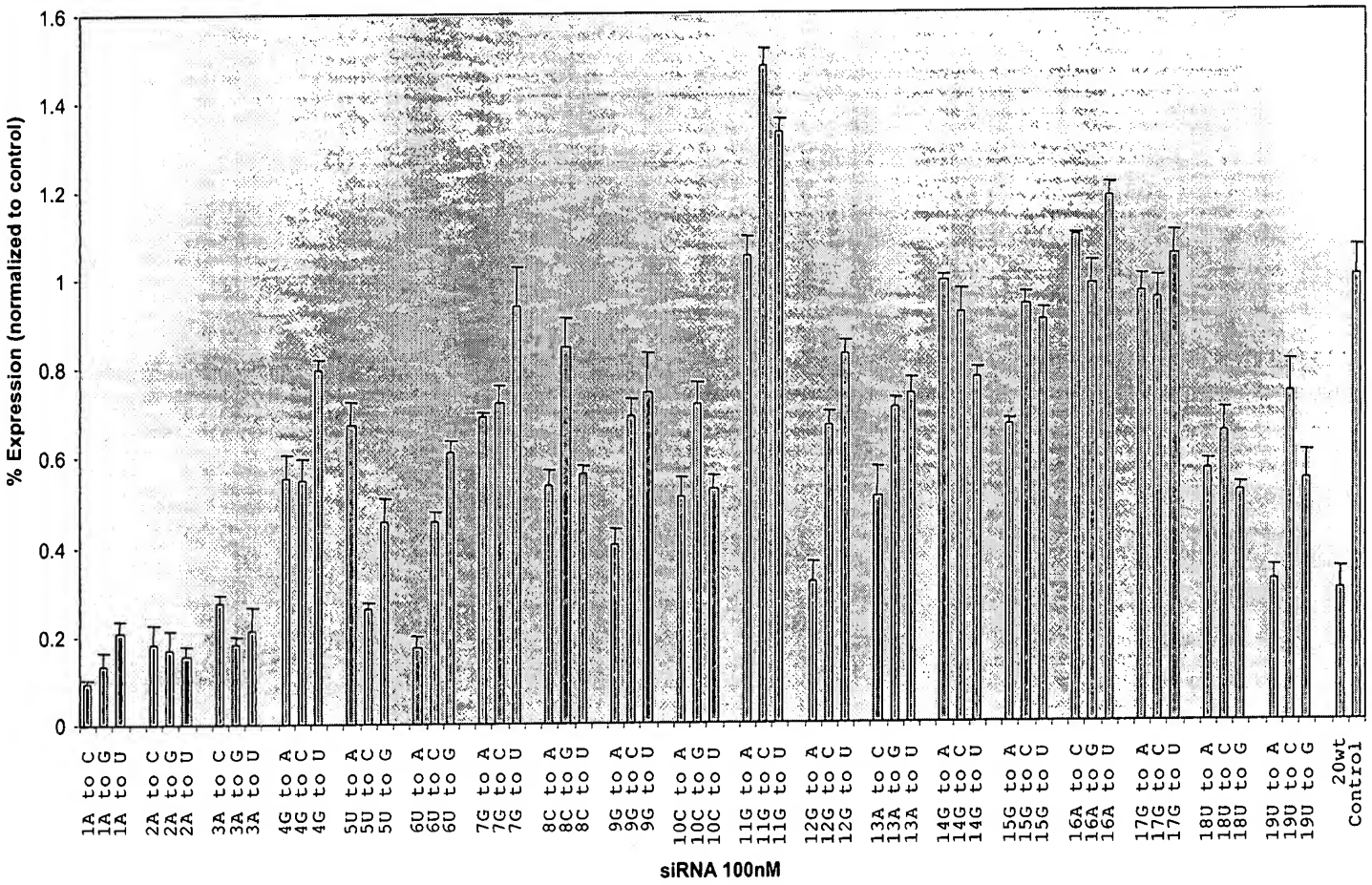
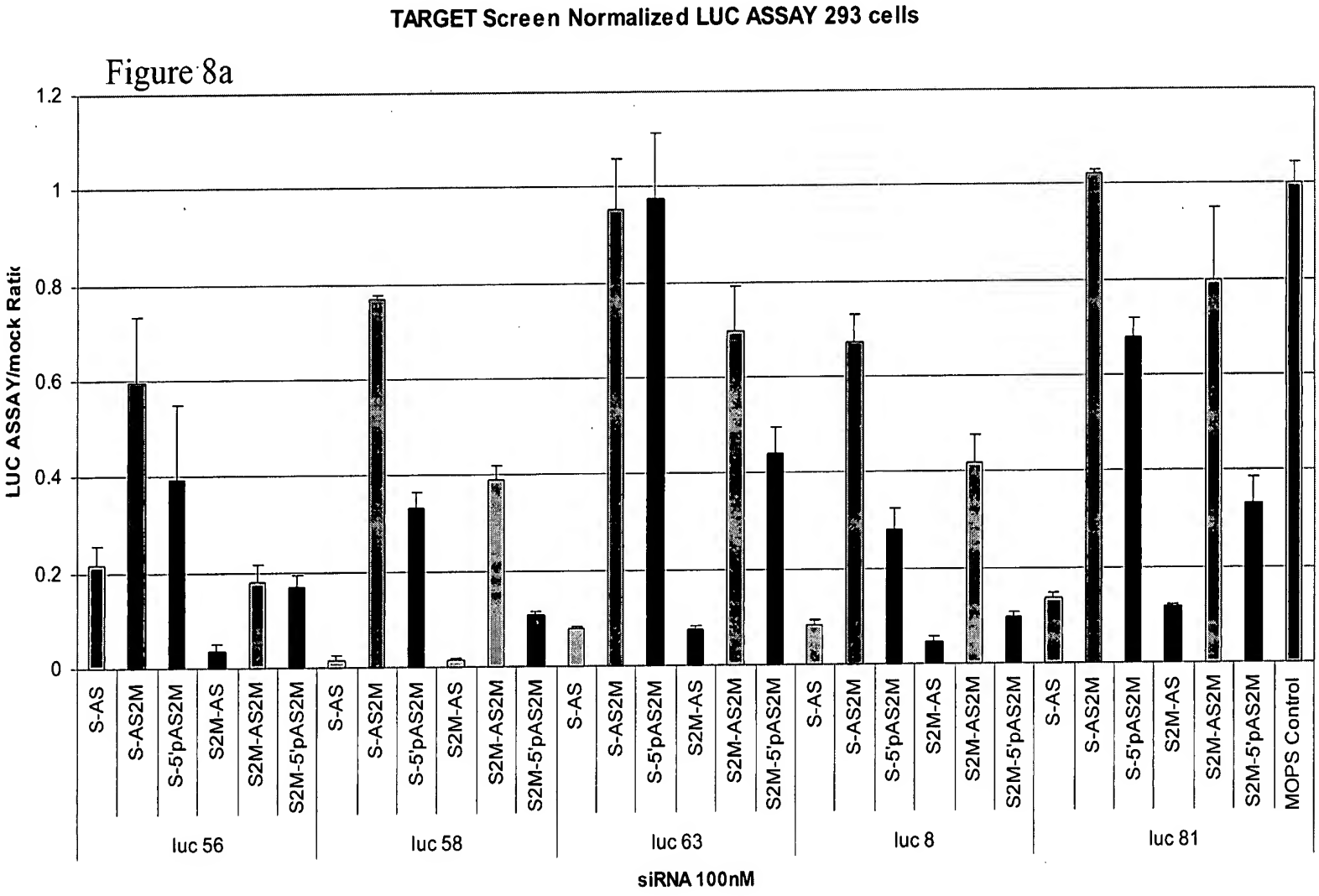

% Expression (normalized to control)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1A to C
1A to G
1A to U
2A to C
2A to G
2A to U
3A to C
3A to G
3A to U
4G to A
4G to C
4G to U
5U to A
5U to C
5U to G
6U to A
6U to C
6U to G
7G to A
7G to C
7G to U
8C to A
8C to G
8C to U
9G to A
9G to C
9G to U
10C to A
10C to G
10C to U
11G to A
11G to C
11G to U
12G to A
12G to C
12G to U
13A to C
13A to G
13A to U
14G to A
14G to C
14G to U
15G to A
15G to C
15G to U
16A to C
16A to G
16A to U
17G to A
17G to C
17G to U
18U to A
18U to C
18U to G
19U to A
19U to C
19U to G
20wt Control
siRNA 100nM

Figure 8a


TARGET Screen Normalized LUC ASSAY 293 cells
LUC ASSAY/mock Ratio
1.2
1
0.8
0.6
0.4
0.2
0
S-AS
S-AS2M
S-5'pAS2M
S2M-AS
S2M-AS2M
S2M-5'pAS2M
luc 56
luc 58
luc 63
luc 8
luc 81
MOPS Control
siRNA 100nM

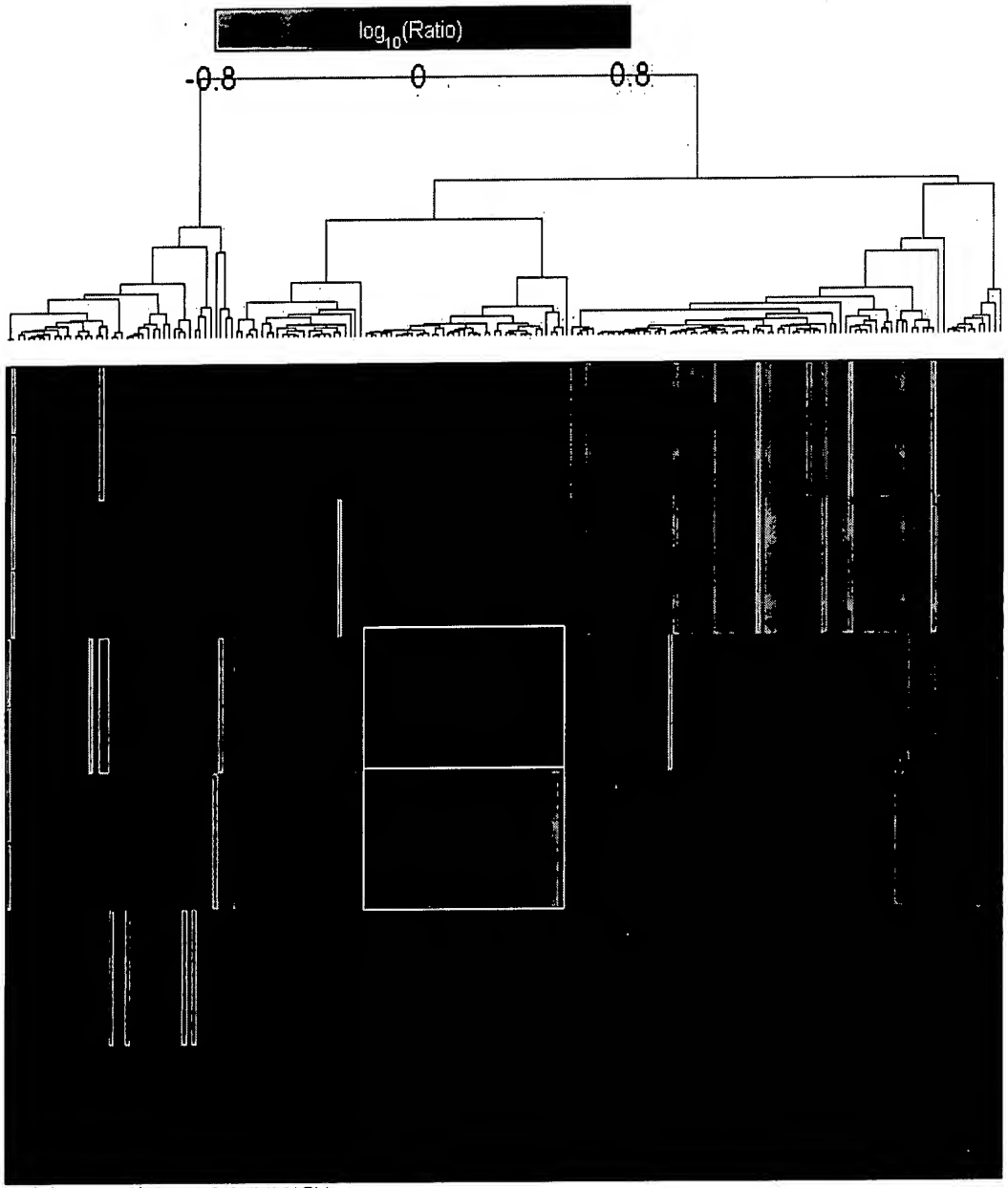


Figure 8b

IGF1R-3

Sense strand modified

IGF1R-3

CLUSTER ANALYSIS SUMMARY:
14-Aug-2003 13:54:57
EG from \\FILER\Bio-public\JSchetter\RNAi\Dharmacon\2003 July\IGF1R chem mod1.mat
Clustering parameters: algorithm = anc, type = gene, similarity measure = correlation-based, metric type = , linka
Cuts: |log10(ratio)| > 0.2 and p-value < 0.01 in 1 or more experiments and with 1 or more genes
Statistics: 193 genes in 6 experiments selected from 23661 genes in 6 experiments

Figure 9

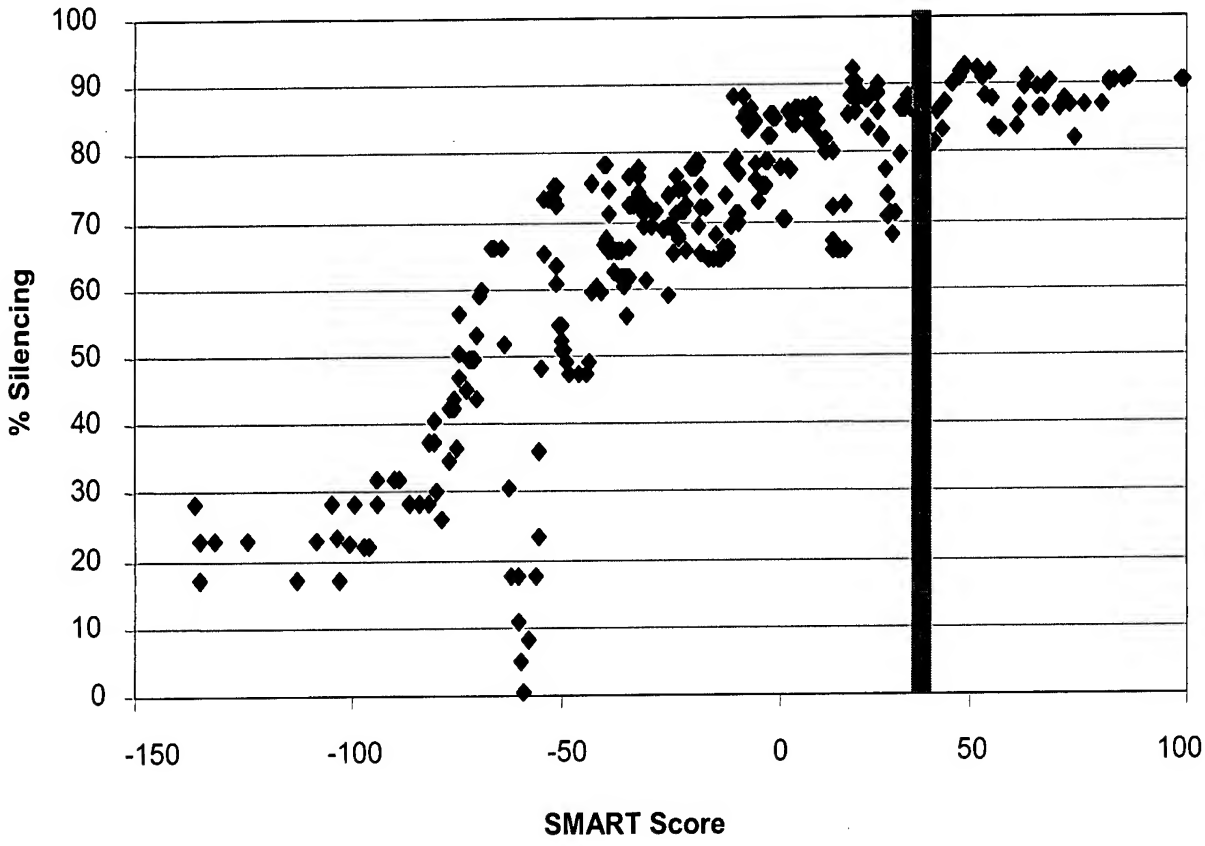

100
90
80
70
60
50
40
30
20
10
0
% Silencing
-150
-100
-50
0
50
100
SMART Score

Figure 10a-f

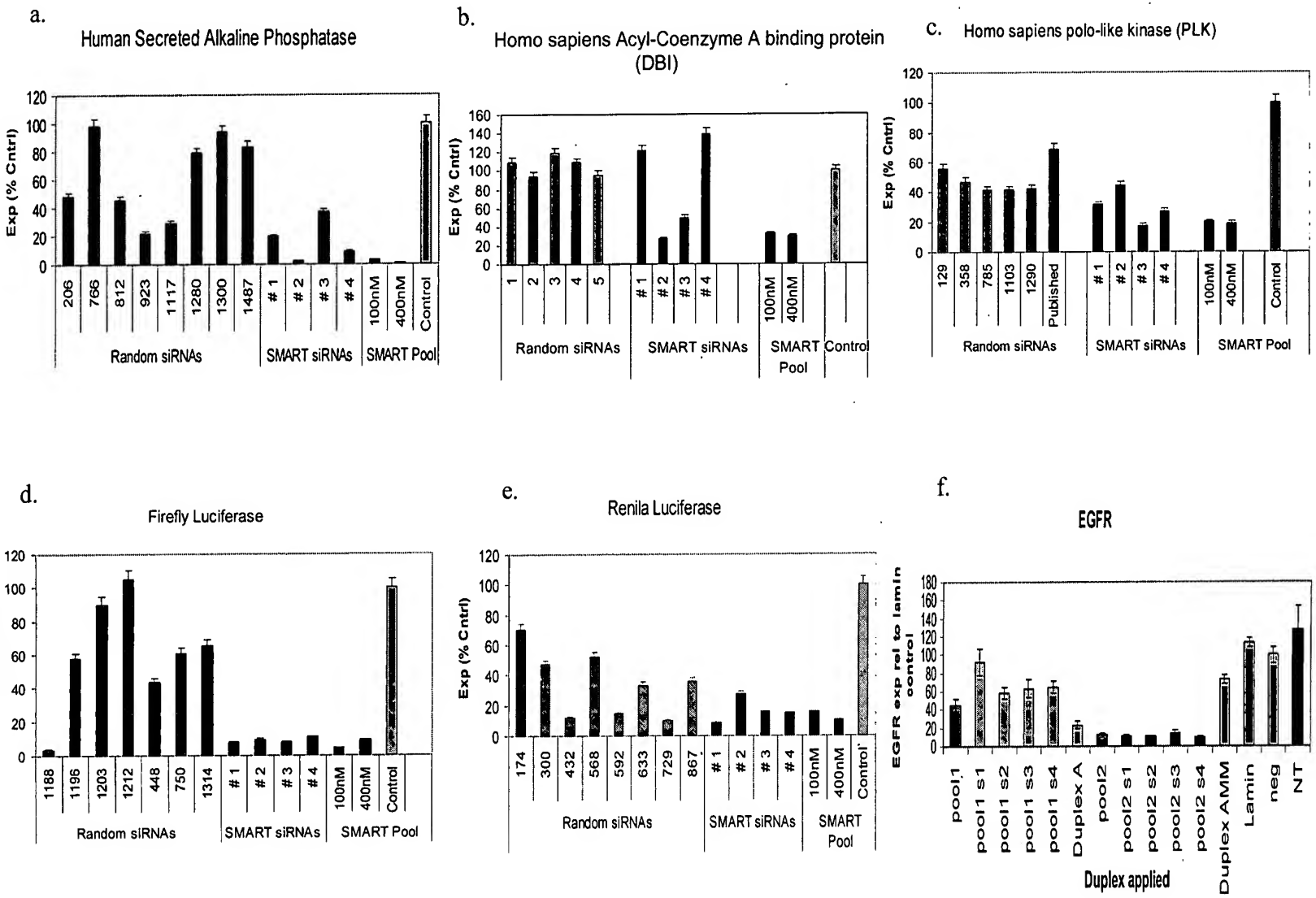

a.
Human Secreted Alkaline Phosphatase
Exp (% Cntrl)
206
766
812
923
1117
1280
1300
1487
1
2
3
4
100nM
400nM
Control
Random siRNAs
SMART siRNAs
SMART Pool
b.
Homo sapiens Acyl-Coenzyme A binding protein (DBI)
Exp (% Cntrl)
1
2
3
4
5
1
2
3
4
100nM
400nM
Control
Random siRNAs
SMART siRNAs
SMART Pool
c.
Homo sapiens polo-like kinase (PLK)
Exp (% Cntrl)
129
358
785
1103
1290
Published
1
2
3
4
100nM
400nM
Control
Random siRNAs
SMART siRNAs
SMART Pool
d.
Firefly Luciferase
Exp (% Cntrl)
1188
1196
1203
1212
448
750
1314
1
2
3
4
100nM
400nM
Control
Random siRNAs
SMART siRNAs
SMART Pool
e.
Renila Luciferase
Exp (% Cntrl)
174
300
432
568
592
633
729
867
1
2
3
4
100nM
400nM
Control
Random siRNAs
SMART siRNAs
SMART Pool
f.
EGFR
EGFR exp rel to lamin control
pool1
pool1 s1
pool1 s2
pool1 s3
pool1 s4
Duplex A
pool2
pool2 s1
pool2 s2
pool2 s3
pool2 s4
Duplex AMM
Lamin
neg
NT
Duplex applied

Figure 11

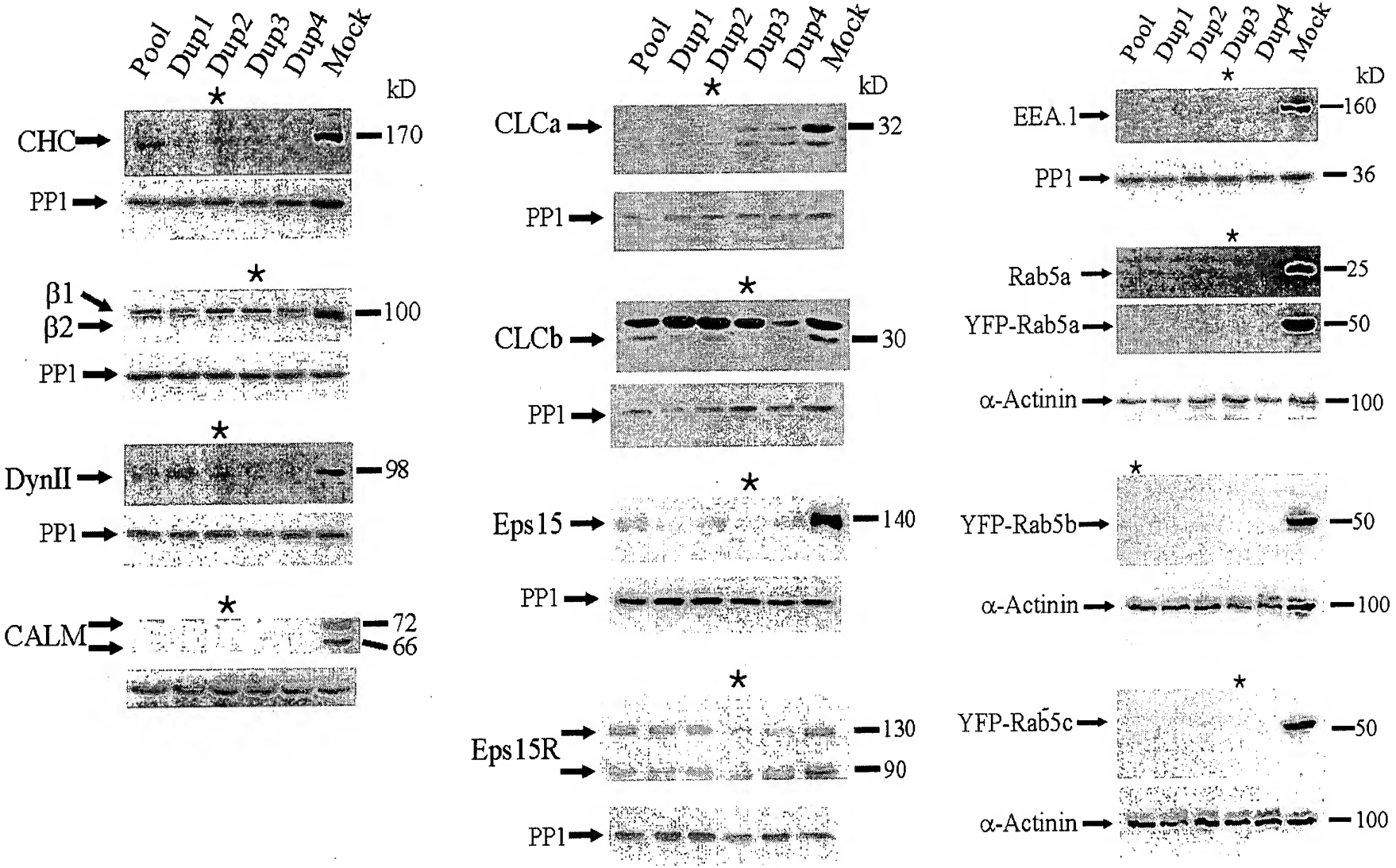

Pool
Dup1
Dup2
Dup3
Dup4
Mock
kD
CHC
170
PP1
β1
β2
100
PP1
DynII
98
PP1
CALM
72
66
CLCa
32
PP1
CLCb
30
PP1
Eps15
140
PP1
Eps15R
130
90
PP1
EEA.1
160
PP1
36
Rab5a
25
YFP-Rab5a
50
α-Actinin
100
YFP-Rab5b
50
α-Actinin
100
YFP-Rab5c
50
α-Actinin
100

Figure 12

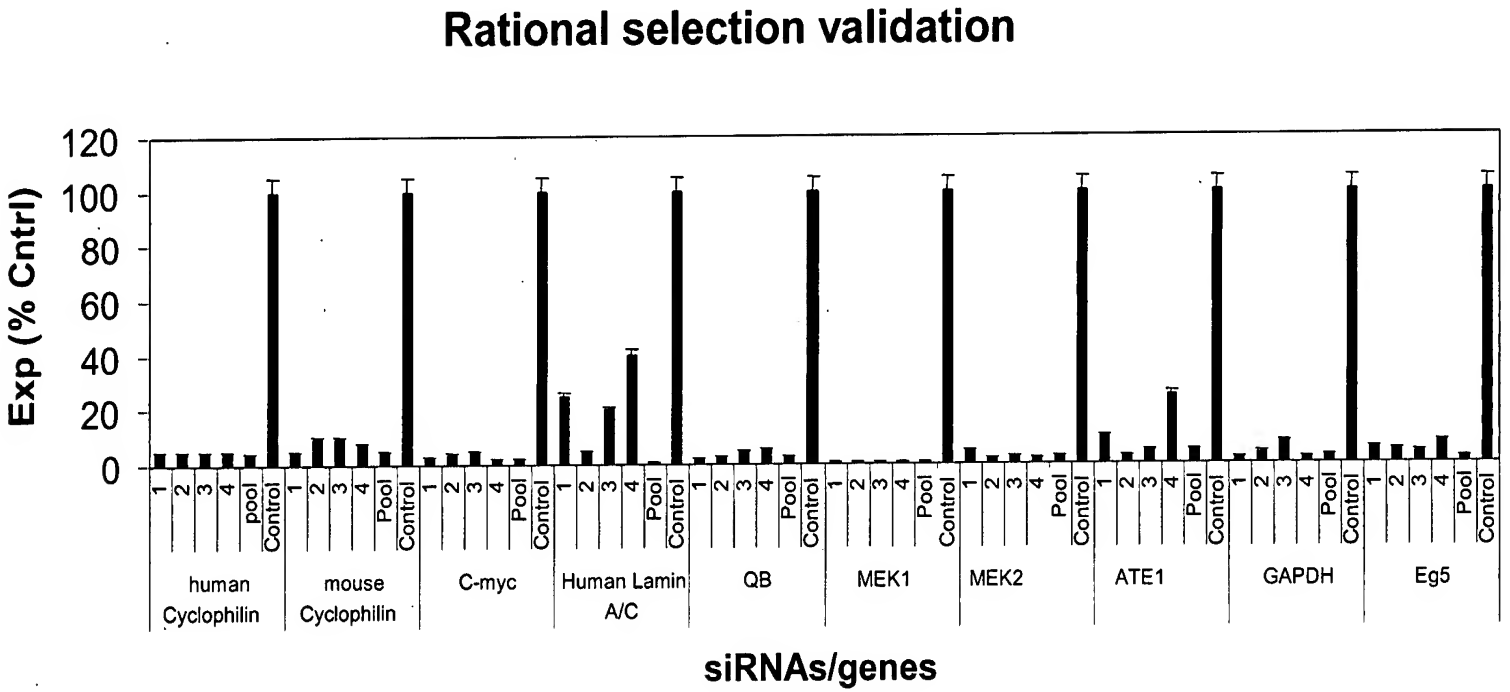
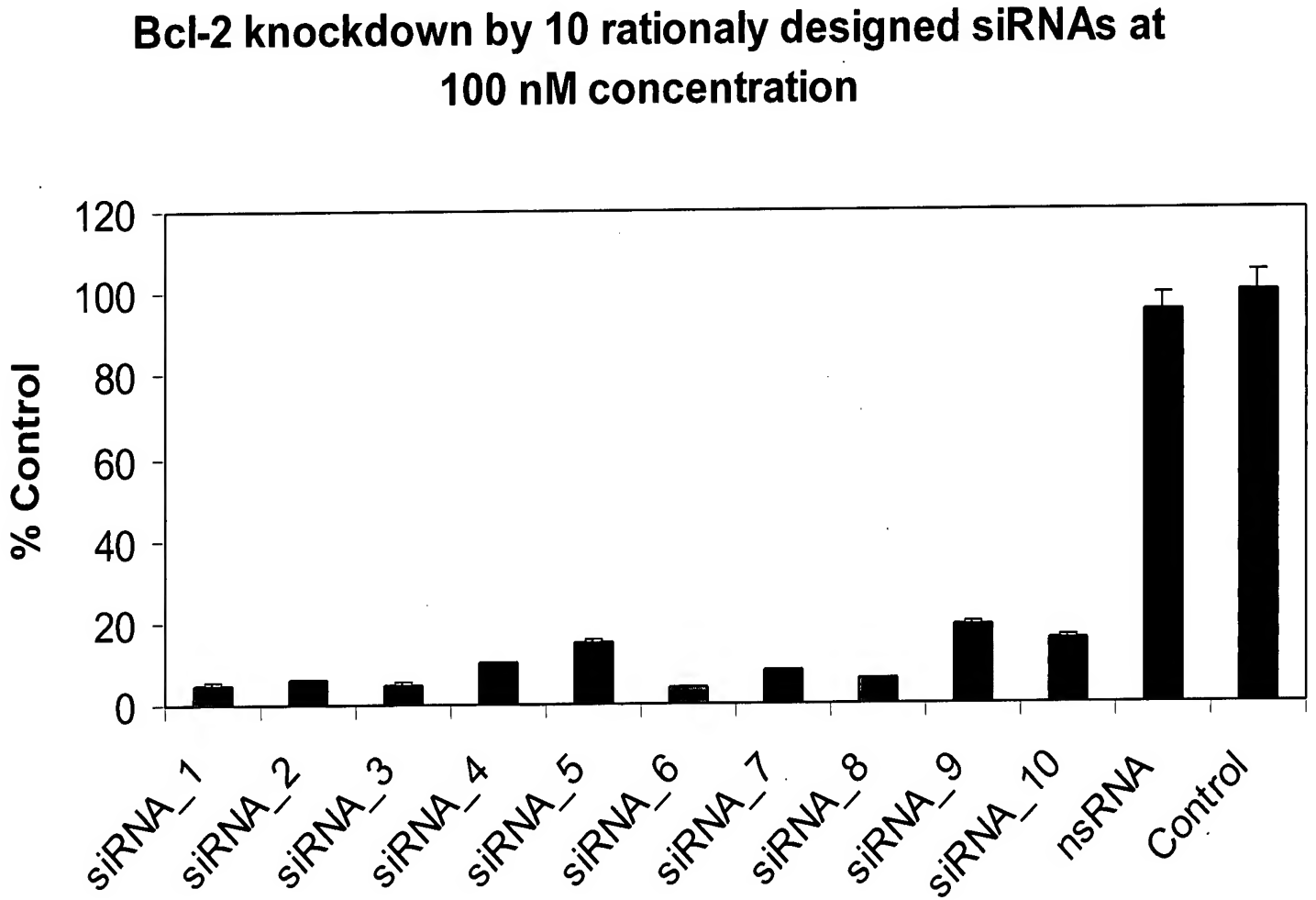

Rational selection validation
Exp (% Cntrl)
120
100
80
60
40
20
0
1 2 3 4 pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
1 2 3 4 Pool Control
human Cyclophilin
mouse Cyclophilin
C-myc
Human Lamin A/C
QB
MEK1
MEK2
ATE1
GAPDH
Eg5
siRNAs/genes

Figure 13 Sequences of top Bcl2

siRNA 1	GGGAGAUAGUGAUGAAGUA
siRNA 2	GAAGUACAUCCAUUAUAAG
siRNA 3	GUACGACAACCGGGAGAUA
siRNA 4	AGAUAGUGAUGAAGUACAU
siRNA 5	UGAAGACUCUGCUCAGUUU
siRNA 6	GCAUGCGGCCUCUGUUUGA
siRNA 7	UGCGGCCUCUGUUUGAUUU
siRNA 8	GAGAUAGUGAUGAAGUACA
siRNA 9	GGAGAUAGUGAUGAAGUAC
siRNA 10	GAAGACUCUGCUCAGUUUG

Figure 14


Bcl-2 knockdown by 10 rationaly designed siRNAs at 100 nM concentration
% Control
120
100
80
60
40
20
0
siRNA_1
siRNA_2
siRNA_3
siRNA_4
siRNA_5
siRNA_6
siRNA_7
siRNA_8
siRNA_9
siRNA_10
nsRNA
Control

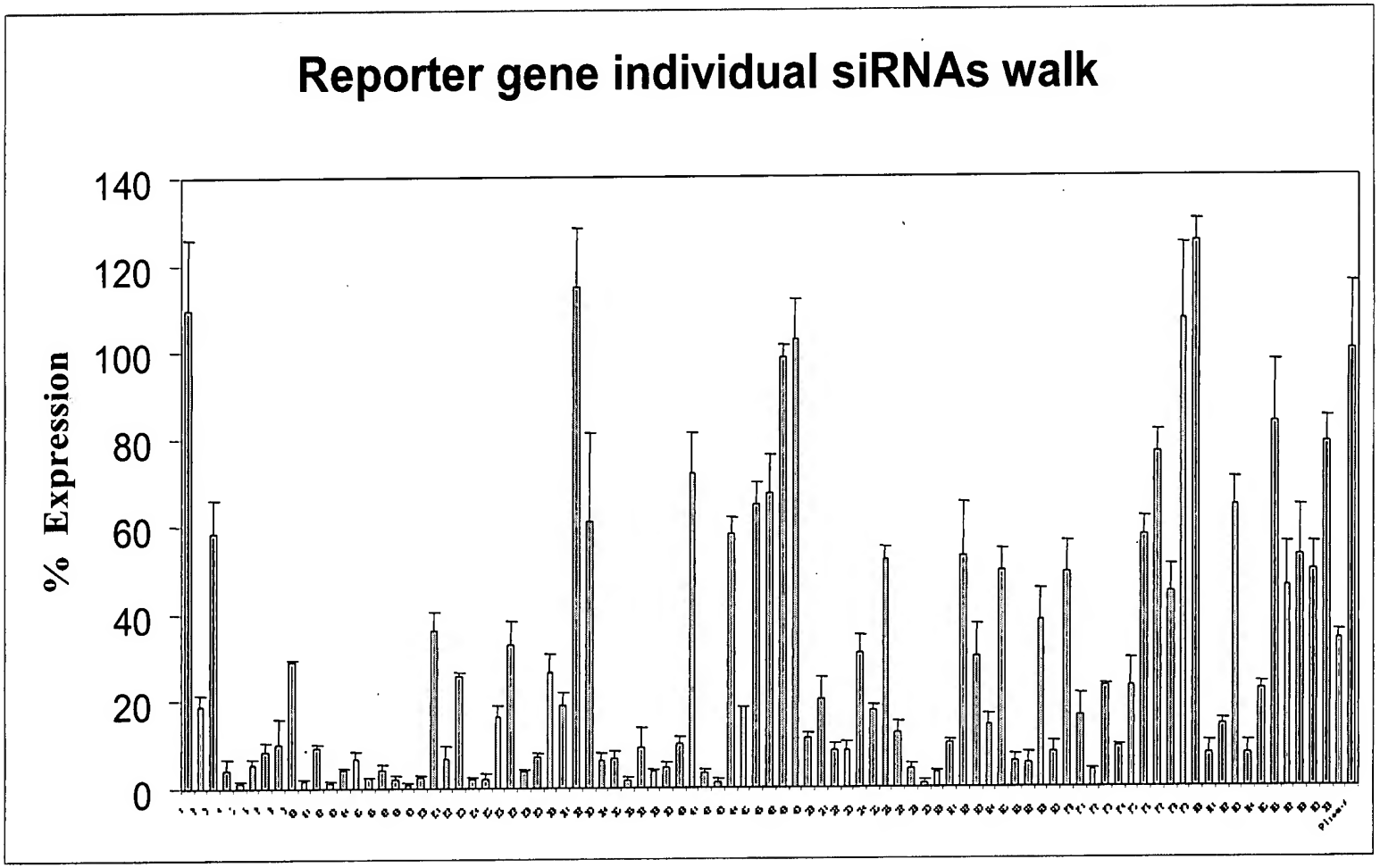

Reporter gene individual siRNAs walk
% Expression
140
120
100
80
60
40
20
0

Figure 15

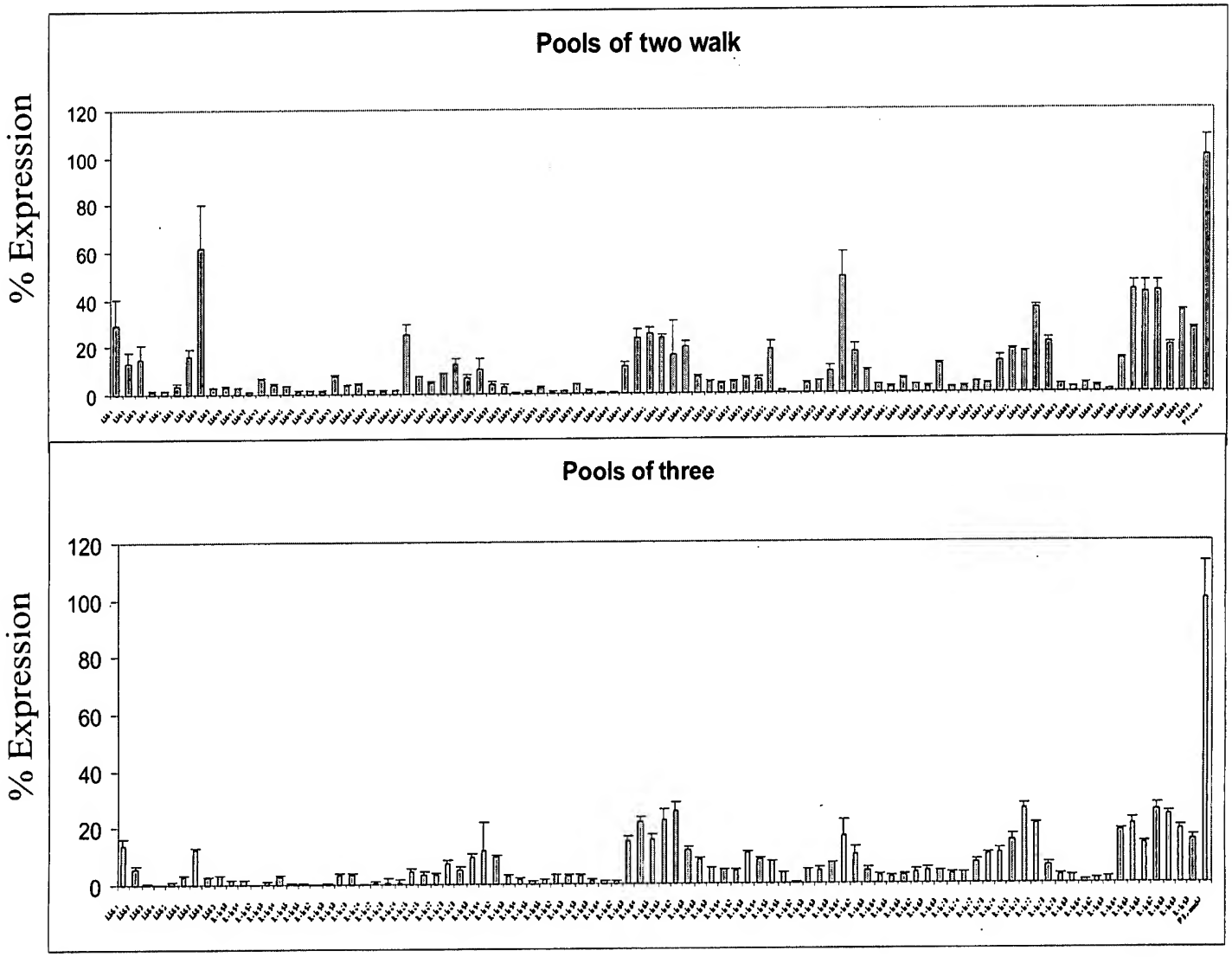

Pools of two walk
% Expression
120
100
80
60
40
20
0
Pools of three
% Expression
120
100
80
60
40
20
0

Figure 16

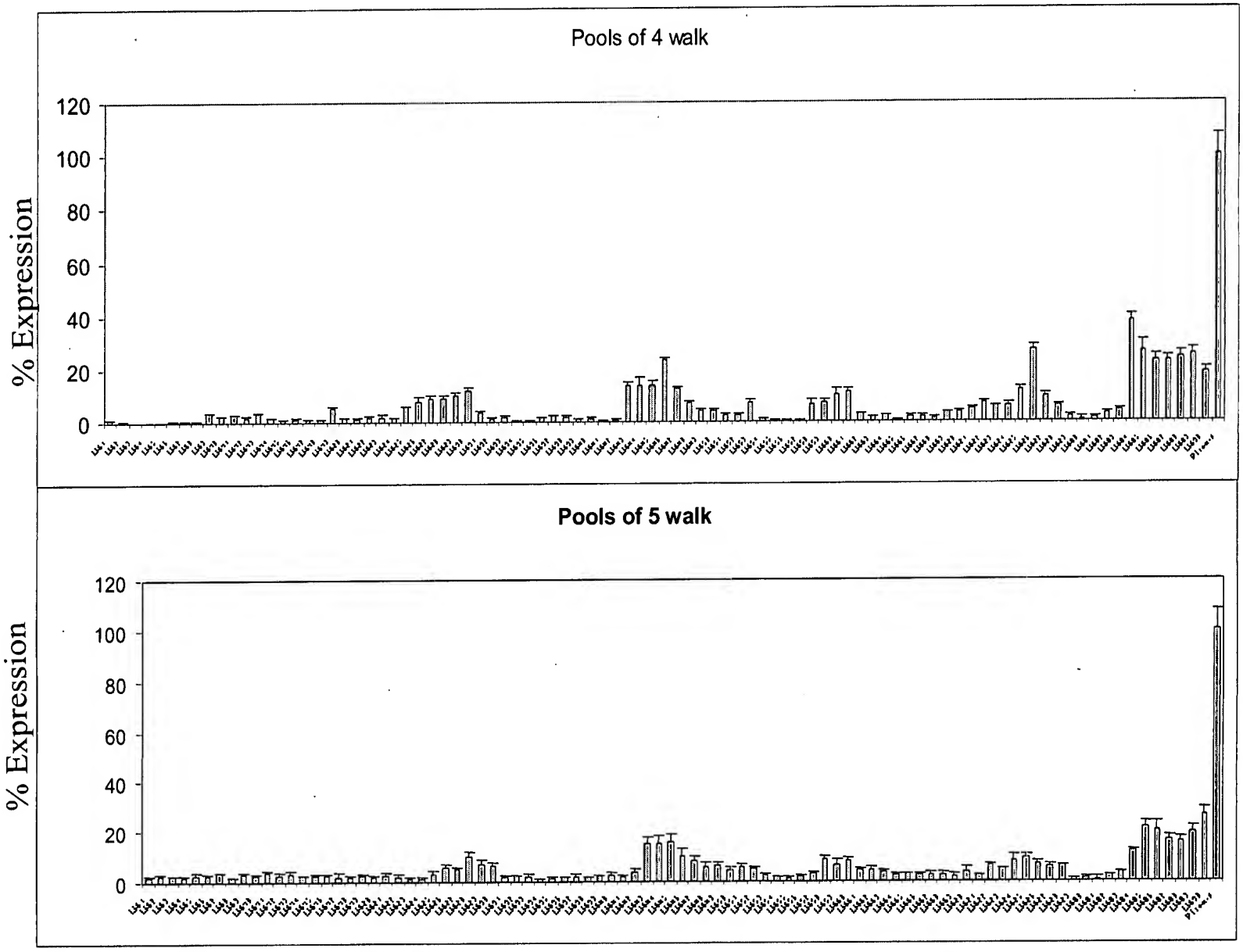

Pools of 4 walk
% Expression
120
100
80
60
40
20
0
Pools of 5 walk
% Expression
120
100
80
60
40
20
0

Figure 17

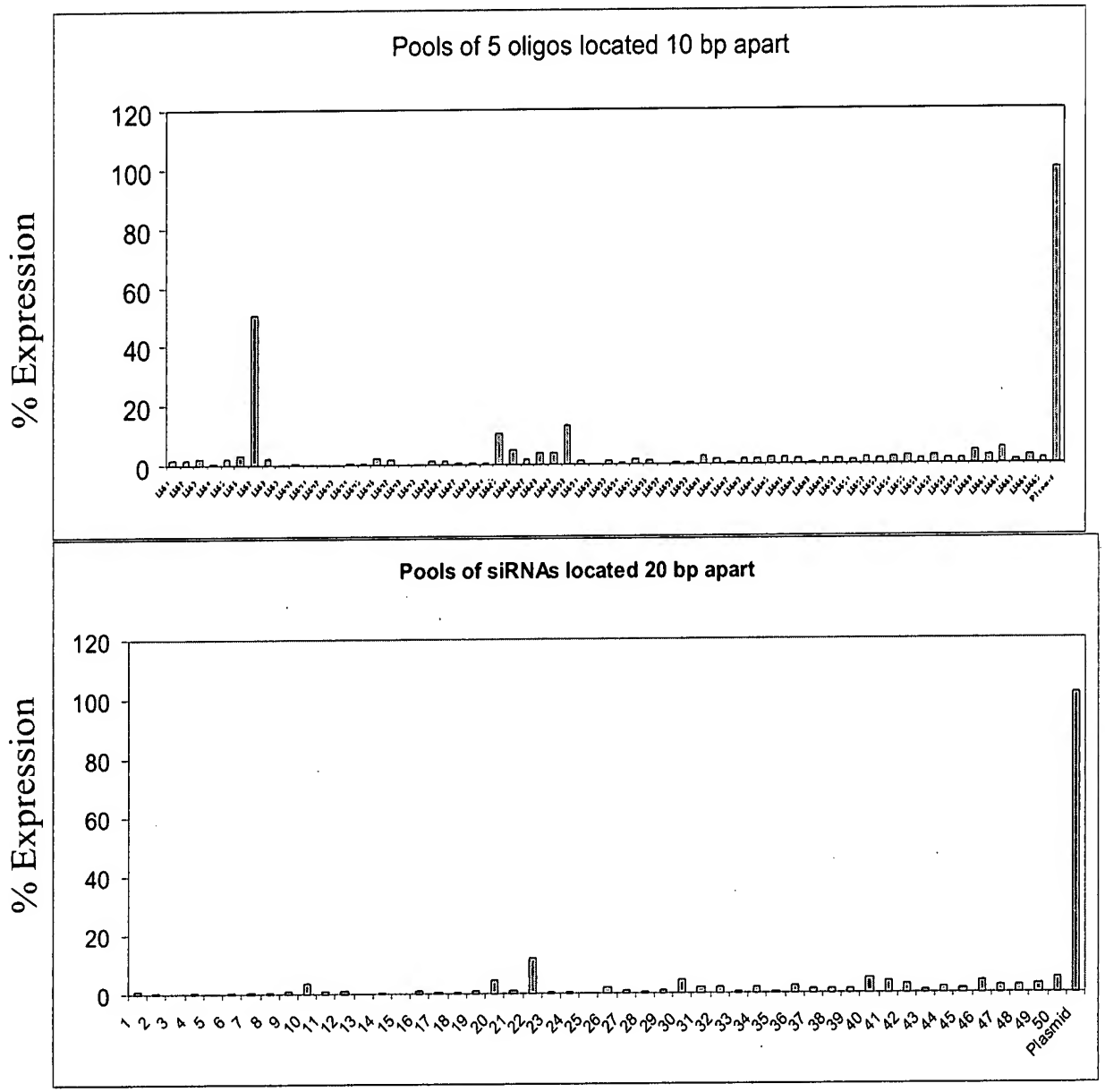

Pools of 5 oligos located 10 bp apart
% Expression
120
100
80
60
40
20
0
Pools of siRNAs located 20 bp apart
% Expression
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 Plasmid

Figure 18

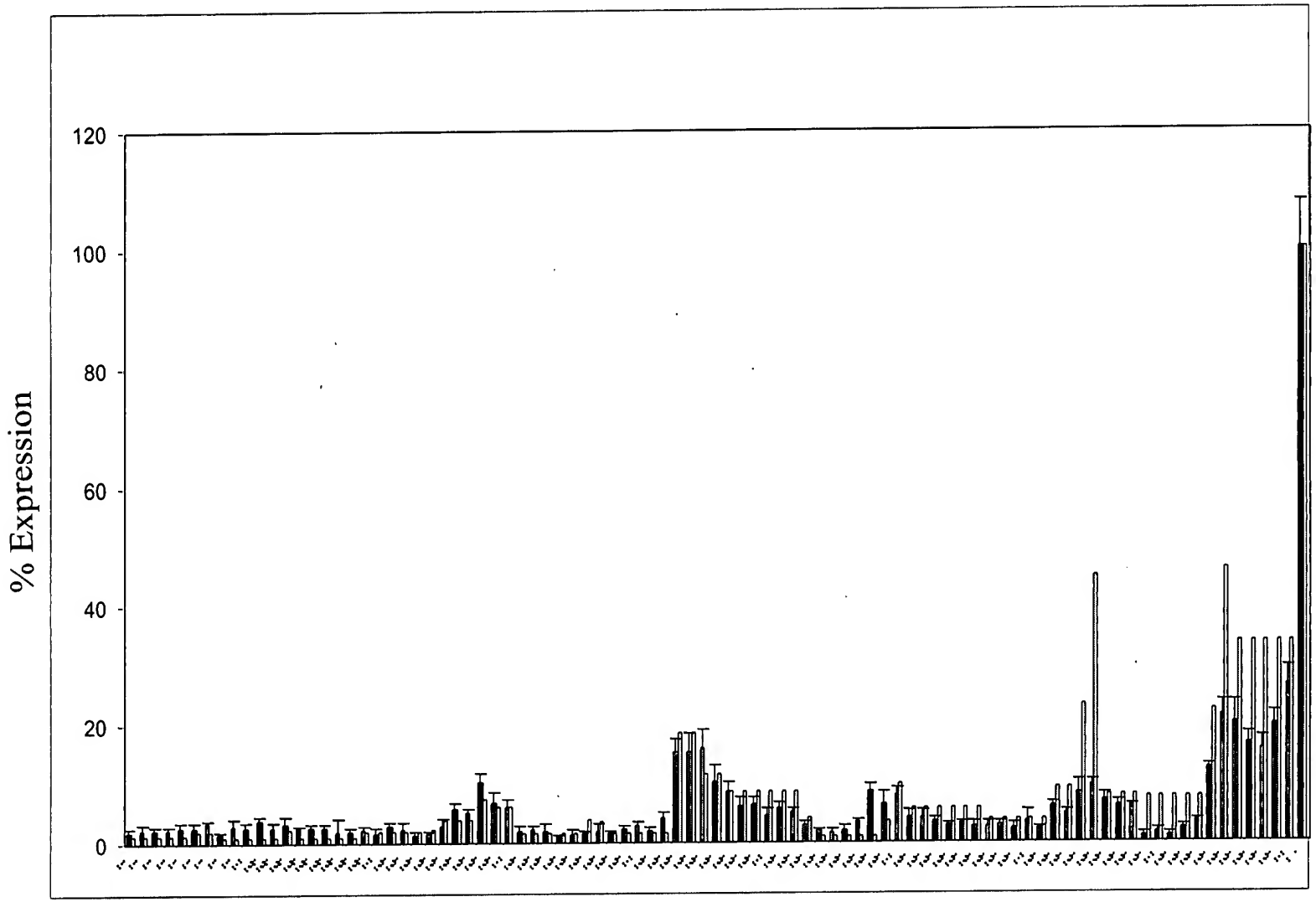


Figure 19

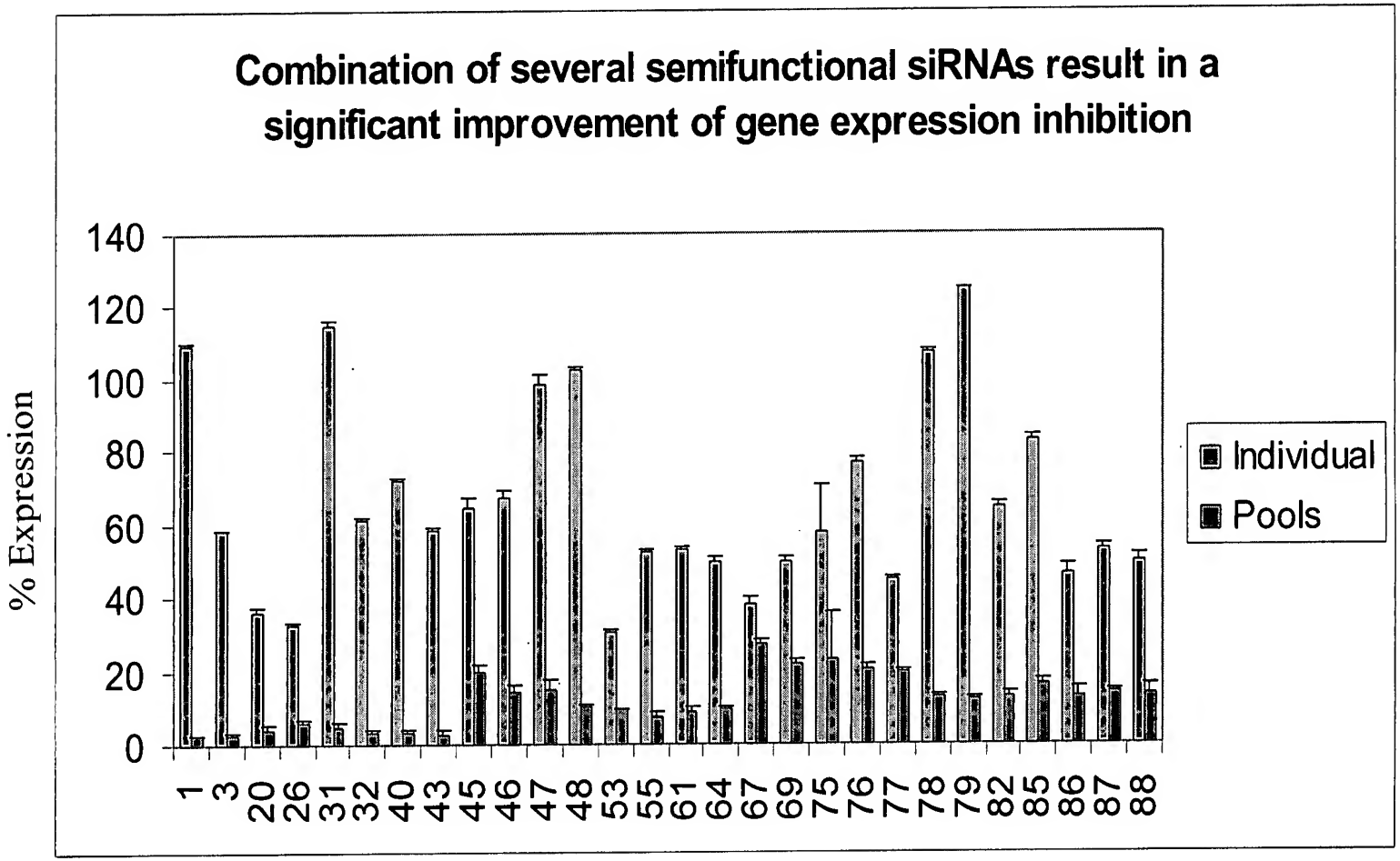
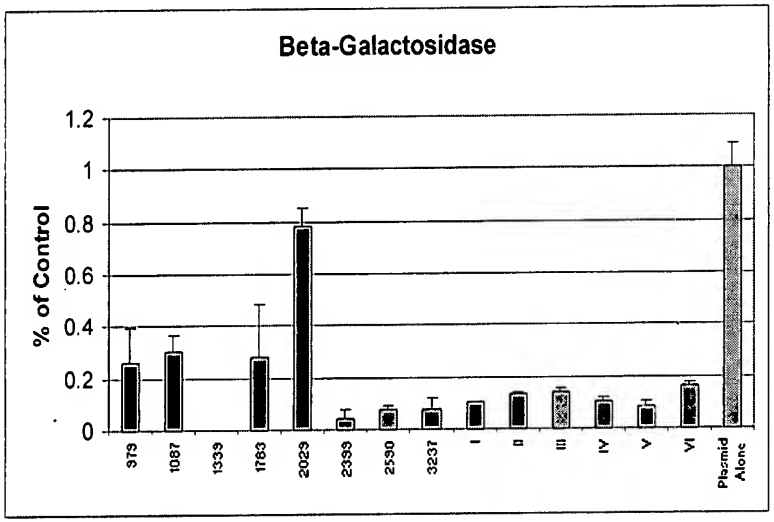
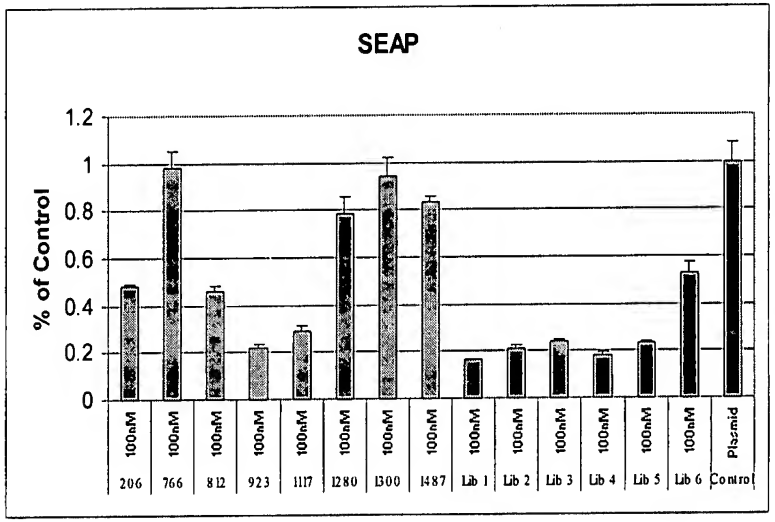

Combination of several semifunctional siRNAs result in a significant improvement of gene expression inhibition
% Expression
140
120
100
80
60
40
20
0
1
3
20
26
31
32
40
43
45
46
47
48
53
55
61
64
67
69
75
76
77
78
79
82
85
86
87
88
Individual
Pools

Figure 20


Beta-Galactosidase
% of Control
1.2
1
0.8
0.6
0.4
0.2
0
979
1087
1339
1763
2023
2393
2590
3237
I
II
III
IV
V
VI
Plasmid Alone


SEAP
% of Control
1.2
1
0.8
0.6
0.4
0.2
0
100nM 206
100nM 766
100nM 812
100nM 923
100nM 1117
100nM 1280
100nM 1300
100nM 1487
100nM Lib 1
100nM Lib 2
100nM Lib 3
100nM Lib 4
100nM Lib 5
100nM Lib 6
Plasmid Control

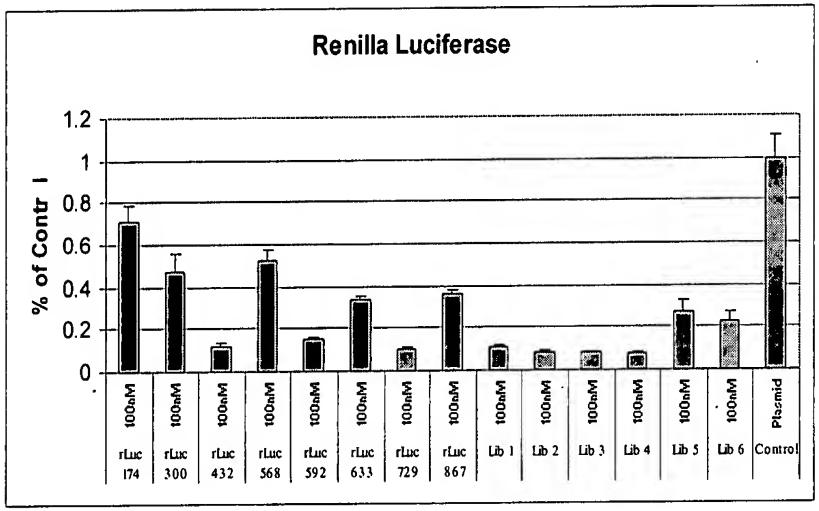

Renilla Luciferase
% of Contr l
1.2
1
0.8
0.6
0.4
0.2
0
100nM rLuc 174
100nM rLuc 300
100nM rLuc 432
100nM rLuc 568
100nM rLuc 592
100nM rLuc 633
100nM rLuc 729
100nM rLuc 867
100nM Lib 1
100nM Lib 2
100nM Lib 3
100nM Lib 4
100nM Lib 5
100nM Lib 6
Plasmid Control

Figure 21

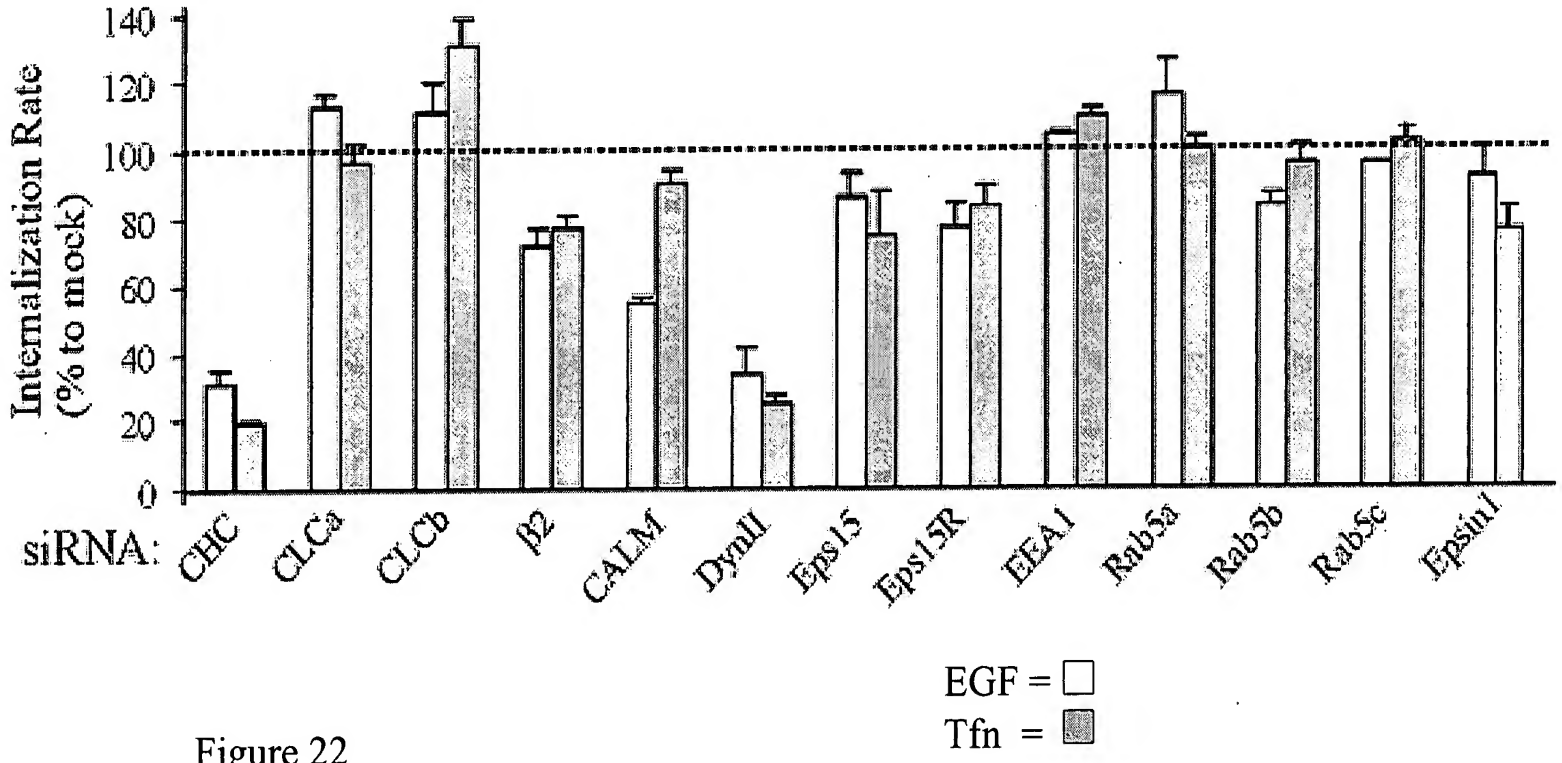

Internalization Rate
(% to mock)
140
120
100
80
60
40
20
0
siRNA:
CHC
CLCa
CLCb
β2
CALM
DynII
Eps15
Eps15R
EEA1
Rab5a
Rab5b
Rab5c
Epsin1
EGF = □
Tfn = ▒

Figure 22

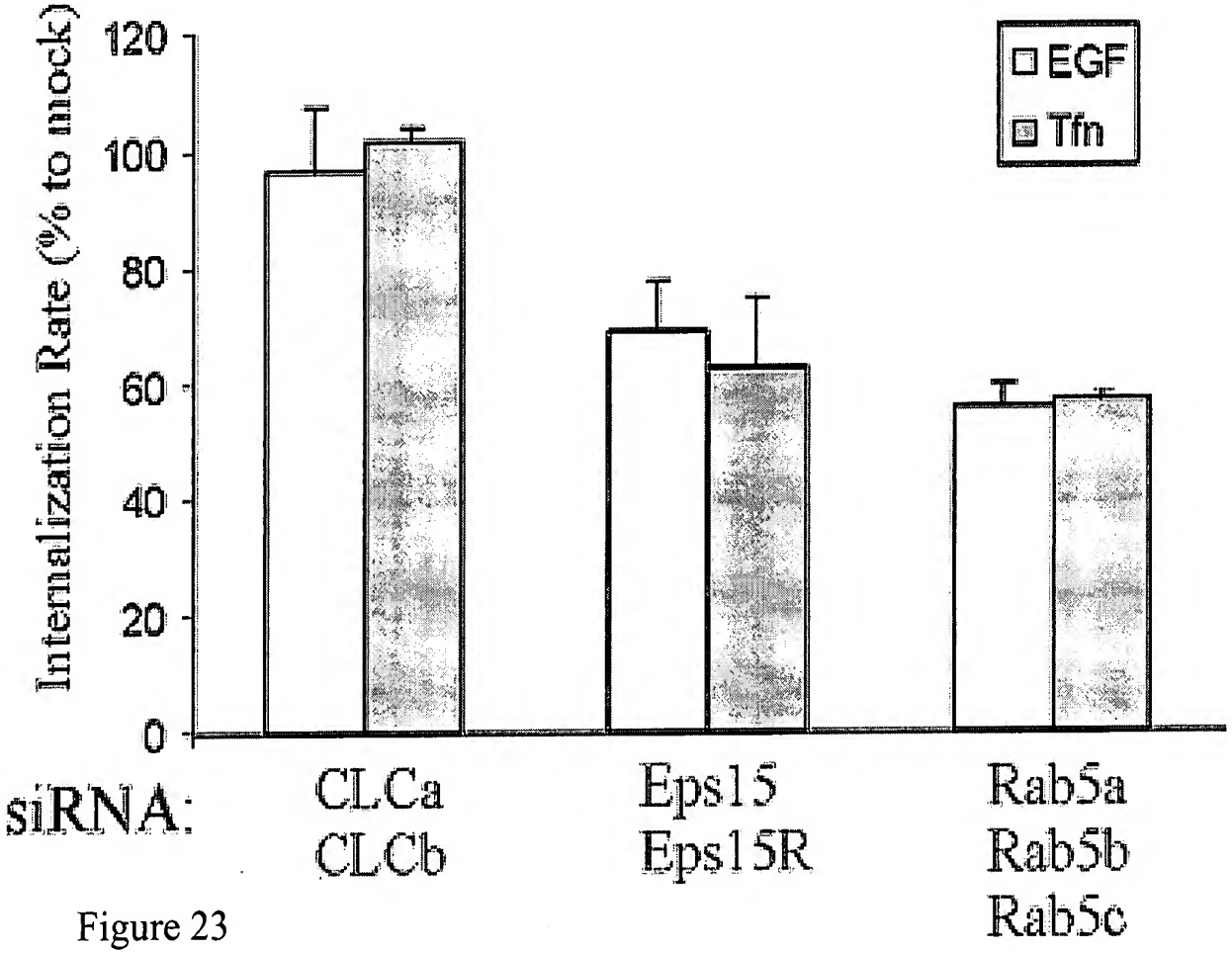

Internalization Rate (% to mock)
120
100
80
60
40
20
0
EGF
Tfn
siRNA:
CLCa
CLCb
Eps15
Eps15R
Rab5a
Rab5b
Rab5c

Figure 23

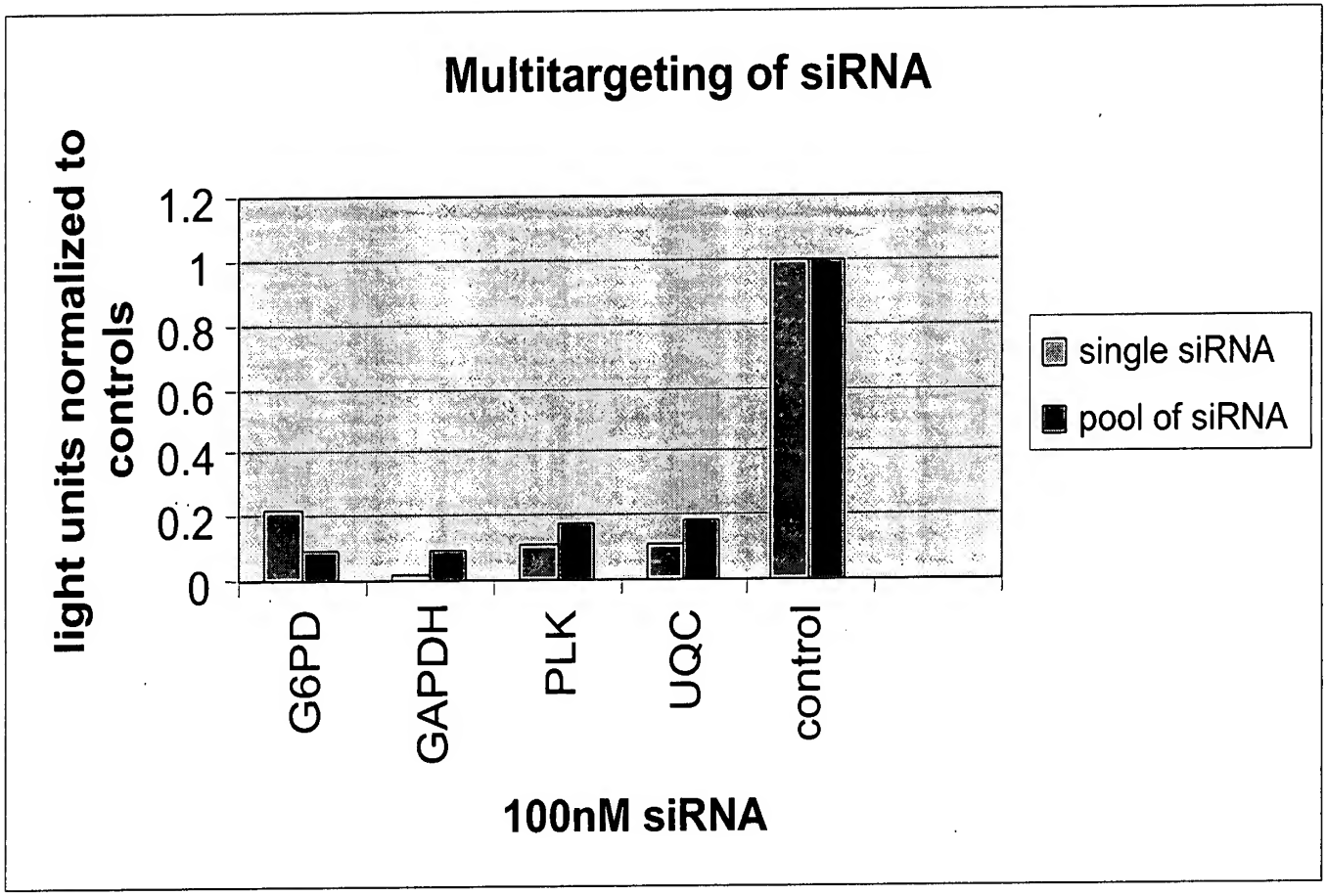
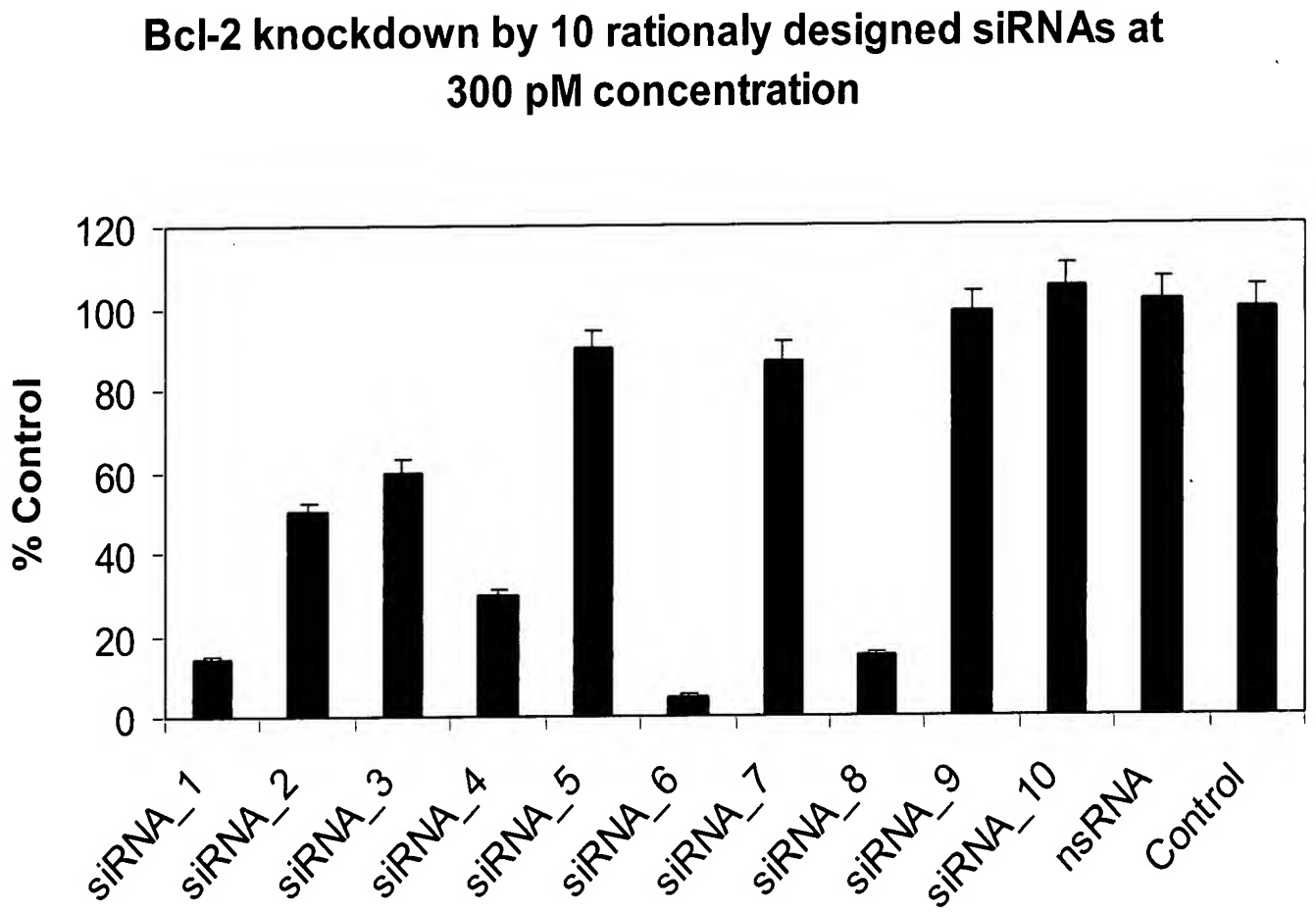

Multitargeting of siRNA
light units normalized to controls
1.2
1
0.8
0.6
0.4
0.2
0
G6PD
GAPDH
PLK
UQC
control
100nM siRNA
single siRNA
pool of siRNA

Figure 24

Figure 25


Bcl-2 knockdown by 10 rationaly designed siRNAs at 300 pM concentration
% Control
120
100
80
60
40
20
0
siRNA_1
siRNA_2
siRNA_3
siRNA_4
siRNA_5
siRNA_6
siRNA_7
siRNA_8
siRNA_9
siRNA_10
nsRNA
Control